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Journal of Chromatography A, 905 (2001) 157–162

JOURNAL OF CHROMATOGRAPHY A

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# Determination of metal complexes of ethylenediaminetetraacetate in the presence of organic matter by high-performance liquid chromatography

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Received 25 July 2000; received in revised form 12 September 2000; accepted 13 September 2000

**Abstract**

A high-performance liquid chromatography method is described here for the determination of the Cd(II), Co(II), Cu(II), Pb(II), and Zn(II) complexes of ethylenediaminetetraacetate (EDTA) in municipal wastewaters and surface waters. The method involves separation by ion-exchange chromatography on a reversed-phase $C_{18}$ column coated with ion-pair reagent, followed by post-column conversion to $FeEDTA^-$ and subsequent detection by UV absorbance. Although Co(II) and Cu(II) coelute, they can be quantified by analyzing absorbance by $CuEDTA^{2-}$ prior to post-column conversion. The method detection limit of $6–8\times10^{-8}$ *M* (5–7 ng) is an order of magnitude improvement over previous UV absorbance post-column reaction methods. The technique can be used in the presence of organic matter encountered in matrices such as untreated wastewater without pre-concentration or sample cleanup. 

*Keywords:* Water analysis; Environmental analysis; Detection, LC; Metal complexes; Ethylenetetraaccetate

## 1. Introduction

Ethylenediaminetetraacetate (EDTA) is the most widely employed of the aminopolycarboxylic acids [1], a class of synthetic chelating agents commonly used in industrial and commercial processes. EDTA is used to complex metal ions in electroplating, pulp and paper processes, leather manufacture, and textile finishing. It is also used in consumer products, such as shampoo, and to protect food from spoilage [2].

Following their use, metal–EDTA complexes are commonly discharged to municipal sewer systems. EDTA is not removed during wastewater treatment [3–6], and has been detected in municipal wastewater at concentrations as high as 19 $\mu M$ [3–8]. While EDTA does not pose a significant risk to human health or ecological systems [2], its ability to complex metals can have an adverse effect on metal removal in wastewater treatment plants. Once released to the environment, EDTA increases the mobility of metals in aquatic systems [8–12]. Complexation by EDTA also substantially reduces the toxicity of pollutant metals to aquatic organisms [13–16]. Due to the potential effects of EDTA on metal fate during treatment and after discharge, sensitive analytical techniques are needed for determining metal–EDTA complexes in environmental samples.

Numerous chromatographic methods have

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0021-9673/01/$ – see front matter 
PII: S0021-9673(00)00964-X

employed EDTA in the mobile phase to separate metal cations during total metals determination [17–19]. This approach has been modified to quantify metal–EDTA complexes in aqueous samples [20,21]. Although EDTA complexes of Fe(III), Cu(II), and Pb(II) can be measured by UV absorption [20], other metal–EDTA complexes exhibit little absorbance above 230 nm. For these metals, post-column reaction has been used to convert the EDTA complexes into $CuEDTA^{2-}$, followed by absorbance detection at 250 nm [20]. This method yields detection limits ranging from 30 to 50 ng for the Zn(II), Cd(II), and Co(II) complexes of EDTA. Unfortunately, concentrations of these complexes in environmental samples are often below this detection limit. Furthermore, the separation technique used in this method, ion-pair chromatography, is subject to significant interference from organic matter, such as that encountered in municipal wastewater and many surface waters.

A second chromatographic method for quantifying metal–EDTA complexes employs the formation of fluorescent ternary complexes in a post-column reaction [21]. The method has a detection limit (i.e. 2 ng) that is an order of magnitude lower than the $CuEDTA^{2-}$ post-column reaction method. This method uses anion-exchange chromatography to separate the complexes on a reversed-phase $C_{18}$ column coated with ion-pair agent. This creative approach results in better separation of the metal–EDTA complexes from coeluting organic matter. Unfortunately, fluorescent organic matter (e.g. humic substances) still interferes with detection of metal–EDTA complexes in surface waters. Furthermore, the presence of $Ca^{2+}$ and $Mg^{2+}$ at concentrations typically encountered in natural waters causes an intense interference peak, which can only be removed by additional cleanup steps.

To determine the speciation of EDTA in environmental samples, a robust and sensitive method is needed that is not adversely affected by organic matter. In this paper, we present a new method that uses high-performance ion-exchange chromatography on an ion-pair agent-coated $C_{18}$ column to separate the complexes, followed by post-column conversion of all species into $FeEDTA^{-}$, which is detected by UV absorbance at 258 nm. Since $FeEDTA^{-}$ has a maximum molar extinction coefficient which is 3–4 times greater than that of $CuEDTA^{2-}$, sensitivity of the analysis is greatly improved over methods which employ post-column conversion of complexes into $CuEDTA^{2-}$. Anion-exchange chromatography on an ion-pair agent-coated $C_{18}$ column results in resolution of the metal–EDTA complexes and avoids interference attributable to organic matter.

## 2. Experimental

### 2.1. Apparatus

High-performance liquid chromatography was performed with a Gynkotek HPLC system consisting of a M480 solvent delivery pump and a Gynkotek Gina 50 autosampler with a variable injection capacity from 1 to 250 μl (250 μl injections were used for sample analysis). Solvents and mobile phases were degassed using an ERC-3315 on-line degasser (ERC). A Gynkotek UVD170S UV–Visible diode array detector was used (258 nm) for analyte detection. The column used was a 25 cm×4.6 mm $C_{18}$ column packed with 5 μm endcapped, metal-free particles (Supelco Discovery), with a 2-cm guard column of the same material. Chromatograms were recorded using the Gynkotek Chromeleon software system. Peak areas were used for quantification.

Prior to its initial use for this method, the HPLC system was passivated to remove trace metals from the system. The passivation technique consisted of pumping a 20% nitric acid solution through the system for 30 min. Passivation was followed by pumping deionized water through the system for 3 h, followed by acetonitrile for 1 h, and the mobile phase for 3 h.

The post-column reagent was delivered by a model 100A pump (Beckman). The reagent stream was connected to the effluent stream by low-pressure PTFE tubing (Supelco), merged at a stainless-steel mixing tee (Valco). The post-column reactor consisted of 6 m of 0.8 mm I.D. spiraled PTFE tubing.

### 2.2. Reagents

All reagent solutions were prepared by dissolving chemicals of the highest available purity in deionized

water purified by a Nanopure II apparatus (Barnstead). All solvents were HPLC grade from Fisher Scientific. Cetyltrimethylammonium bromide (cetrimide) was obtained from Aldrich. All other chemicals were obtained from Fisher Scientific.

EDTA stock solutions were prepared by dissolving equimolar amounts of metal salt and disodium EDTA in water and diluting as required. The mobile phase consisted of 6 m*M* $NaSO_4$ and 1 m*M* 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, adjusted to pH 7 with $NH_4OH$. The post-column reagent was prepared by adding 40 μ*M* $Fe(NO_3)_3$ to a 200-m*M* sodium formate–formic acid buffer at pH 4.5.

### 2.3. Chromatography

Prior to analysis, the column was coated with cetrimide by pumping a 1 m*M* cetrimide, methanol–water (30:70) solution through the column at 0.5 ml/min for 10 h. The column was then washed with water for 30–60 min before switching to the mobile phase. Analytical conditions are summarized in Table 1. For analysis, the HPLC flow-rate was 1.5 ml/min, while the post-column delivery pump was operated at 0.3 ml/min. To remove the cetrimide from the column after analysis, an acetonitrile–water gradient program was run from 50 to 95% acetonitrile at a constant rate over 3 h.

### 2.4. Sample collection

All bottles, filters, and tubing used for sample collection or storage were made of PTFE, polypropylene, or polyethylene. Wastewater samples were collected from the Southeast San Francisco (SESF) municipal wastewater treatment plant on 2–3 August, 1999, using trace metal clean procedures [22]. Samples were filtered at the time of collection through a 0.45 μm on-line filter (MSI). Samples were stored at 4°C prior to analysis, which generally occurred within 24 h of collection.

## 3. Results and discussion

### 3.1. Separation of EDTA complexes

We initially attempted to use ion-pair chromatography with cetrimide and high proportions of organic solvent for complex separation, but could not achieve adequate separations of $CuEDTA^{2-}$ and

Table 1
General conditions

| | |
|---|---|
| Chromatography | |
| Guard column | Discovery LC-18, 4.0 mm I.D. (Supelco) |
| Analytical column | Discovery LC-18, 25 cm, 4.6 mm I.D. (Supelco) |
| Pretreatment | System passivation |
| | Column precoating with cetrimide |
| Eluent | 6 m*M* $Na_2SO_4$ |
| | 1 m*M* HEPES, adjusted to pH 7 with $NH_4OH$ |
| Flow rate | 1.5 ml/min |
| Sample loop | 250 μl |
| Run time | 30 min |
| Post-column reaction | |
| Reagent | 50 m*M* sodium formate–150 m*M* formic acid |
| | 40 m*M* $Fe(NO_3)_3$ |
| Reagent flow-rate | 0.3 ml/min |
| Reaction coil | 6 m of 0.8 mm I.D. PTFE tubing |
| Reaction temperature | Room temperature |
| UV detection | |
| Cell volume | 10 μl |
| Path length | 9 mm |
| Wavelength | 258 nm |

$ZnEDTA^{2-}$. In addition, organic matter in the wastewater samples often caused interference peaks coincident with the metal–EDTA complexes and severe baseline drift during analysis. These problems were ameliorated by the use of a $C_{18}$ column coated with ion-pair agent. The coated column acted as an anion-exchange column, without retaining metals in the system as might occur with a cation-exchange column. By preventing buildup of metals on surfaces, which could perturb metal–EDTA speciation during analysis [7], the speciation of metal–EDTA complexes was preserved during analysis.

Variations in the mobile phase concentration of sulfate altered the retention time of the EDTA complexes, but not their relative retention times (i.e. the ratio of the retention factors was unchanged). For example, changing the concentration of sulfate from 6 m*M* to 12 m*M* reduced the retention time for $ZnEDTA^{2-}$ from ~28 to 6 min. However, we do not recommend the modification of the mobile phase to reduce retention times because the stationary phase tends to clog in the presence of high sulfate concentrations.

A typical chromatogram for a mixture of EDTA complexes is shown in Fig. 1. Under these conditions, the retention factors (i.e. *k*) are 10.7 (Cd), 13.1 (Pb), 18.5 (Co), 18.9 (Cu), and 20.2 (Zn). Attempts to improve the separation by decreasing the post-column reaction time were unsuccessful. The rate of the post-column reaction did not increase significantly by heating the post-column reactor to 40°C. In addition, the pH of the post-column reagent could not be reduced because a complex exhibiting a lower molar extinction coefficient (i.e. FeHEDTA) forms at lower pH values.

As indicated by these *k* values, this method cannot fully resolve $CuEDTA^{2-}$ and $CoEDTA^{2-}$ (i.e. the peaks overlap) (Fig. 2). However, UV detection can be used to quantify $CuEDTA^{2-}$ without post-column conversion [20], allowing for determination of each of the complexes with two separate injections, if necessary. In the first injection, made without post-column reaction, $CuEDTA^{2-}$ can be detected. A separate injection can then be made with the post-column reactor engaged. This injection can be used to quantify the sum ($CuEDTA^{2-}$+$CoEDTA^{2-}$). $CoEDTA^{2-}$ can then be determined by difference. This procedure exhibits decreased $CuEDTA^{2-}$ sensitivity, but allows for quantification of the complexes separately.

The method is capable of quantifying all of the important metal–EDTA complexes in municipal wastewater and surface water except $FeEDTA^{-}$ and $NiEDTA^{2-}$. $FeEDTA^{-}$ cannot be detected because it has a very low affinity for the stationary phase, relative to sulfate. $NiEDTA^{2-}$ cannot be detected because its slow ligand exchange kinetics prevent it from being converted into $FeEDTA^{-}$ in the post-column reactor. If necessary, these metal–EDTA complexes can be quantified using previously published methods [7,8].

As mentioned previously, it is important that the column remain metal-free to ensure that EDTA speciation does not change during the analysis. To minimize the potential for complex formation or dissociation during analysis, we used an endcapped

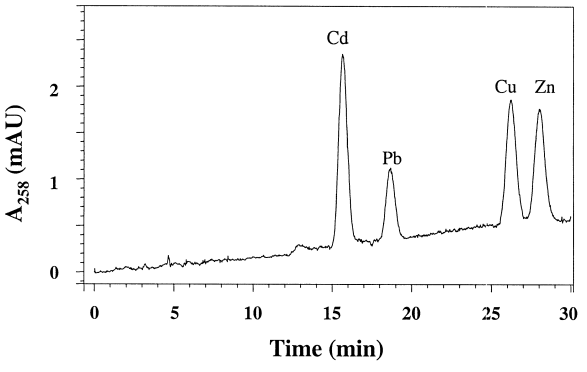


Fig. 1. Chromatogram of a standard containing $CdEDTA^{2-}$ (1.3 μ*M*), $PbEDTA^{2-}$ (0.5 μ*M*), $CuEDTA^{2-}$ (1 μ*M*) and $ZnEDTA^{2-}$ (1 μ*M*).

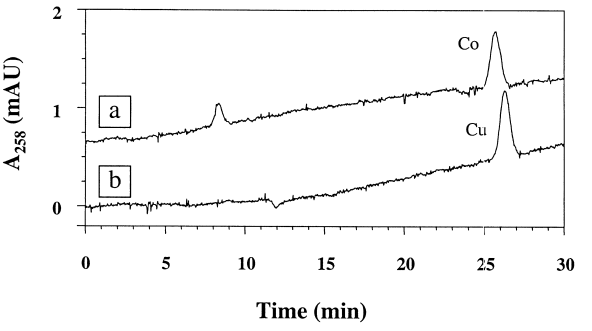


Fig. 2. Chromatograms of standards containing 0.5 μ*M* each of (a) $CoEDTA^{2-}$, (b) $CuEDTA^{2-}$.

column to eliminate surface sites for metal adsorption on the column, as well as polyether ether ketone (PEEK) tubing for all system components which came into contact with the samples. To assess the potential for changes in speciation during analysis, disodium EDTA samples were injected at concentrations up to 1 m*M*. This high concentration of exchangeable EDTA could easily be converted to another form of EDTA (e.g. $ZnEDTA^{2-}$) if any metal contamination existed in the system. In all cases, conversion to other metal–EDTA complexes accounted for less than 5% of the EDTA injected.

### 3.2. Detection of EDTA complexes

Solutions of metal–EDTA complexes varying in concentration from 0.1 to 5 μ*M* were analyzed. Over this concentration range, all of the EDTA complexes exhibited linear calibration graphs with correlation coefficients, $r^2$, ranging from 0.985 to 0.999. The slope of the calibration curve was unchanged between standards and spiked primary wastewater (i.e. settled sewage) samples, indicating that there is no significant change in response, even in the presence of high concentrations of organic matter. UV absorbance detection at 258 n*M*, corresponding to the peak absorbance of $FeEDTA^{-}$, was used to quantify the metal–EDTA complexes. Interference at this wavelength can be overcome by quantifying at wavelengths up to 300 nm without significant decreases in sensitivity: quantification at 300 nm decreased the method sensitivity by ~40%. Detection limits for the analysis at 258 nm, based on a signal-to-noise ratio of 3, were 60–80 n*M* (5–8 ng). This detection limit is in the same range as the previous UV absorbance method for $CuEDTA^{2-}$ and $PbEDTA^{2-}$ [20], but represents nearly an order of magnitude improvement in sensitivity for Co(II), Cd(II), and Zn(II).

### 3.3. EDTA complexes in municipal wastewater

Since our objective in developing this method is to provide a means for the determination of metal–EDTA complexes at the low concentrations and complicated matrices encountered in environmental samples, it is important to assess potential matrix effects. Fig. 3b illustrates a typical chromatogram of primary wastewater. The chromatogram shows little interference, and peaks corresponding to 300 n*M* $CuEDTA^{2-}$ and 310 n*M* $ZnEDTA^{2-}$ are visible. Fig. 3a illustrates the same wastewater sample amended with 500 n*M* $CuEDTA^{2-}$ and $ZnEDTA^{2-}$. Spike recoveries performed in wastewater influent and effluent samples from four different municipal wastewater treatment plants were nearly quantitative (Zn: mean=93%, $n=22$; Cu: mean=89%, $n=12$). Other metal–EDTA complexes (i.e. $CdEDTA^{2-}$, $CoEDTA^{2-}$) were not detected in these samples because the concentrations of these metals are relatively low. It should also be noted that the concentration of $ZnEDTA^{2-}$ in Fig. 3b (310 n*M*) is below the limit of detection for other methods using UV absorption for detection.

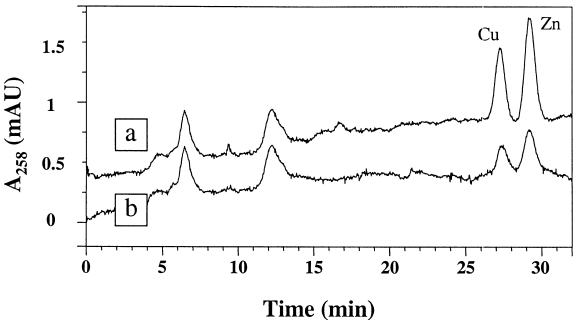


Fig. 3. Chromatograms of primary wastewater effluent collected from the Southeast San Francisco wastewater treatment plant, 2 August 1999: (a) sample spiked with 0.5 μ*M* each $CuEDTA^{2-}$ and $ZnEDTA^{2-}$; (b) sample only.

### 3.4. Other potential applications

In addition to applications in municipal wastewater treatment, the analytical technique presented here has potential applications in environmental and industrial settings. Since this method is capable of detecting pollutant metal–EDTA complexes at low concentrations and without substantial matrix interference, it could be useful in quantifying metal–EDTA complexes in soil, groundwater, and hazardous waste. For example, the method could be used to further the understanding of the important role metal–EDTA complexes play in phytoremediation of metal-contaminated soils [23,24]. In addition, the methods could be adapted to separate and quantify

metal complexes with other synthetic chelating agents. This could be useful in industrial wastewater applications receiving significant amounts of other polycarboxylate chelating agents (e.g. nitrilotriacetate [NTA], diethylenetriaminepentaacetic acid [DTPA]).

## Acknowledgements

This research was supported by the Water Environment Research Foundation. We would like to thank James Hunt and Kenneth Raymond for critical reviews of the manuscript.

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